

10/516908

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(FILE 'HOME' ENTERED AT 09:39:30 ON 25 JAN 2008)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
LIFESCI' ENTERED AT 09:40:05 ON 25 JAN 2008

L1 1 S SITE(W) TARGETED (W) ANTICOAGULANT?
L2 27787 S "ANNEXIN V"
L3 10651 S (FUS? OR ATTCAH? OR BIND?) AND L2
L4 603 S L3 AND ANTICOAGULANT?
L5 0 S TPI AND L4
L6 5303 S KUNITZ (2W) INHIBITOR?
L7 8 S L4 AND L6
L8 2 DUP REM L7 (6 DUPLICATES REMOVED)
L9 10 S L2 AND L6
L10 4 DUP REM L9 (6 DUPLICATES REMOVED)
L11 7748 S (AMYLOID BETA-PROTEIN PRECURSOR?) OR (TICK ANTICOAGULANT PEPT
L12 5 S L2 AND L11
L13 2 DUP REM L12 (3 DUPLICATES REMOVED)
L14 14 S APROTININ AND L2
L15 5 DUP REM L14 (9 DUPLICATES REMOVED)
 E WUN T C/AU
L16 281 S E3
L17 25 S L6 AND L16
L18 9 DUP REM L17 (16 DUPLICATES REMOVED)
L19 2 S L18 AND L2
L20 1 S L18 AND MEMBRANE?
L21 6 S (PS (W) BINDING) AND KUNITZ
L22 1 DUP REM L21 (5 DUPLICATES REMOVED)

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NEWS 3	AUG 06	FSTA enhanced with new thesaurus edition	
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NEWS 5	AUG 20	CA/CAplus enhanced with CAS indexing in pre-1907 records	
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NEWS 7	AUG 27	USPATOLD now available on STN	
NEWS 8	AUG 28	CAS REGISTRY enhanced with additional experimental spectral property data	
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NEWS 11	SEP 13	INPADOCDB enhanced with monthly SDI frequency	
NEWS 12	SEP 17	CA/CAplus enhanced with printed CA page images from 1967-1998	
NEWS 13	SEP 17	CAplus coverage extended to include traditional medicine patents	
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NEWS 15	OCT 02	CA/CAplus enhanced with pre-1907 records from Chemisches Zentralblatt	
NEWS 16	OCT 19	BEILSTEIN updated with new compounds	
NEWS 17	NOV 15	Derwent Indian patent publication number format enhanced	
NEWS 18	NOV 19	WPIX enhanced with XML display format	
NEWS 19	NOV 30	ICSD reloaded with enhancements	
NEWS 20	DEC 04	LINPADOCDB now available on STN	
NEWS 21	DEC 14	BEILSTEIN pricing structure to change	
NEWS 22	DEC 17	USPATOLD added to additional database clusters	
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NEWS 24	DEC 17	DGENE now includes more than 10 million sequences	
NEWS 25	DEC 17	TOXCENTER enhanced with 2008 MeSH vocabulary in MEDLINE segment	
NEWS 26	DEC 17	MEDLINE and LMEDLINE updated with 2008 MeSH vocabulary	
NEWS 27	DEC 17	CA/CAplus enhanced with new custom IPC display formats	
NEWS 28	DEC 17	STN Viewer enhanced with full-text patent content from USPATOLD	
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NEWS EXPRESS 19 SEPTEMBER 2007: CURRENT WINDOWS VERSION IS V8.2,
CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
AND CURRENT DISCOVER FILE IS DATED 19 SEPTEMBER 2007.

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=> file medline embase biosis biotechds scisearch hcplus ntis lifesci
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ENTRY SESSION
FULL ESTIMATED COST 0.21 0.21

FILE 'MEDLINE' ENTERED AT 09:40:05 ON 25 JAN 2008

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=> s site(w)targeted (w)anticoagulant?
L1 1 SITE(W) TARGETED (W) ANTICOAGULANT?

=> d ibib ab

L1 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2003:991276 HCAPLUS
DOCUMENT NUMBER: 140:35943
TITLE: Recombinant fusion of annexin V (ANV) and Kunitz
protease inhibitors (KPI) as novel site-
targeted anticoagulants exhibiting
stronger activities than their components
INVENTOR(S): Wun, Tze Chein
PATENT ASSIGNEE(S): USA
SOURCE: PCT Int. Appl., 59 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003103577	A2	20031218	WO 2003-US17442	20030604
WO 2003103577	A3	20040304		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2486362	A1	20031218	CA 2003-2486362	20030604
AU 2003237351	A1	20031222	AU 2003-237351	20030604
AU 2003237351	A2	20031222		
EP 1509541	A2	20050302	EP 2003-736814	20030604
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
CN 1659180	A	20050824	CN 2003-812999	20030604
JP 2005528124	T	20050922	JP 2004-510698	20030604
US 2005164926	A1	20050728	US 2005-516908	20050317
PRIORITY APPLN. INFO.:			US 2002-386932P	P 20020606
			WO 2003-US17442	W 20030604

AB Novel recombinant anticoagulation proteins, methods of their use and methods of their production are described. In particular, recombinant fusions of annexin V (ANV) and Kunitz protease inhibitors (KPI) that possess potent anticoagulant activity are provided. The fusions, abbreviated ANV:KPI, utilize ANV having high affinity for phosphatidyl-L-serine with various KPI's to target serine proteases in membrane-associated coagulation complexes in the blood coagulation cascade. ANV:KPIs are potentially useful antithrombotic drugs permitting localized passivation of thrombogenic vessel walls and associated thrombi.

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L1 1 S SITE(W) TARGETED (W) ANTICOAGULANT?

=> S "annexin V"

L2 27787 "ANNEXIN V"

=> S (fus? or attcah? or bind?) and l2

L3 10651 (FUS? OR ATTCAH? OR BIND?) AND L2

=> S l3 and anticoagulant?

L4 603 L3 AND ANTICOAGULANT?

=> S TPI and l4

L5 0 TPI AND L4

=> S kunitz (2w) inhibitor?

L6 5303 KUNITZ (2W) INHIBITOR?

=> S l4 and l6

L7

8 L4 AND L6

=> dup rem 17
PROCESSING COMPLETED FOR L7
L8 2 DUP REM L7 (6 DUPLICATES REMOVED)

=> d 1-2 ibib ab

L8 ANSWER 1 OF 2 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2005231329 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15677561
TITLE: Fusion proteins comprising annexin
V and Kunitz protease inhibitors
are highly potent thrombogenic site-directed
anticoagulants.
AUTHOR: Chen Hsiao-Hui; Vicente Cristina P; He Li; Tollesen Douglas
M; Wun Tze-Chein
CORPORATE SOURCE: Division of Hematology, Department of Medicine, Washington
University School of Medicine, St Louis, MO, USA.
CONTRACT NUMBER: R01 HL55520 (NHLBI)
SOURCE: Blood, (2005 May 15) Vol. 105, No. 10, pp. 3902-9.
Electronic Publication: 2005-01-27.
Journal code: 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, N.I.H., EXTRAMURAL)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200506
ENTRY DATE: Entered STN: 4 May 2005
Last Updated on STN: 8 Jun 2005
Entered Medline: 7 Jun 2005

AB The anionic phospholipid, phosphatidyl-L-serine (PS), is sequestered in the inner layer of the plasma membrane in normal cells. Upon injury, activation, and apoptosis, PS becomes exposed on the surfaces of cells and sheds microparticles, which are procoagulant. Coagulation is initiated by formation of a tissue factor/factor VIIa complex on PS-exposed membranes and propagated through the assembly of intrinsic tenase (factor VIIIa/factor IXa), prothrombinase (factor Va/factor Xa), and factor XIa complexes on PS-exposed activated platelets. We constructed a novel series of recombinant anticoagulant fusion proteins by linking annexin V (ANV), a PS-binding protein, to the Kunitz-type protease inhibitor (KPI) domain of tick anticoagulant protein, an aprotinin mutant (6L15), amyloid beta-protein precursor, or tissue factor pathway inhibitor. The resulting ANV-KPI fusion proteins were 6- to 86-fold more active than recombinant tissue factor pathway inhibitor and tick anticoagulant protein in an in vitro tissue factor-initiated clotting assay. The in vivo antithrombotic activities of the most active constructs were 3- to 10-fold higher than that of ANV in a mouse arterial thrombosis model. ANV-KPI fusion proteins represent a new class of anticoagulants that specifically target the anionic membrane-associated coagulation enzyme complexes present at sites of thrombogenesis and are potentially useful as antithrombotic agents.

L8 ANSWER 2 OF 2 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
DUPLICATE 2

ACCESSION NUMBER: 2004-04247 BIOTECHDS
TITLE: New recombinant anticoagulant protein comprising a
fusion annexin V (ANV) and
Kunitz protease inhibitor (KPI), useful for

treating unstable angina, myocardial infarction, aneurysms,
atherosclerosis, thalassemia, thrombosis;
recombinant fusion protein for use in gene
therapy

AUTHOR: WUN T C
PATENT ASSIGNEE: WUN T C
PATENT INFO: WO 2003103577 18 Dec 2003
APPLICATION INFO: WO 2003-US17442 4 Jun 2003
PRIORITY INFO: US 2002-386932 6 Jun 2002; US 2002-386932 6 Jun 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-053570 [05]

AB DERWENT ABSTRACT:

NOVELTY - A recombinant anticoagulant protein comprising a fusion annexin V (ANV) (SEQ ID 10; 319 amino acid sequence defined in the specification) and Kunitz protease inhibitor (KPI), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) an anti-thrombotic composition comprising the recombinant anticoagulant protein; (2) a method of inhibiting blood coagulation in a mammalian subject by administering the recombinant anticoagulant protein to the subject; (3) a method of producing the recombinant anticoagulant protein by linking ANV and KPI; (4) a method of treating or preventing an excess of thrombotic activity in a subject by administering to the subject the anti-thrombotic composition; (5) a recombinant DNA molecule comprising a first DNA sequence encoding ANV and a second DNA sequence encoding KPI; (6) a host cell comprising the recombinant DNA molecule; (7) a stably transfected cell line expressing the recombinant anticoagulant protein; (8) a prokaryotic or eukaryotic cell line; (9) a process for preparing a cell line expressing the recombinant anticoagulant protein by stably transfecting a host cell with the recombinant expression vector; and (10) a recombinant expression vector comprising a first nucleotide sequence encoding ANV to Ala mutation of ANV, or its conservatively substituted variants, and a second nucleotide sequence of KPI together with additional sequences capable of directing the synthesis of the recombinant anticoagulant protein.

BIOTECHNOLOGY - Preferred Protein: The recombinant anticoagulant protein comprises a protein sequence selected from TAP-ANV (SEQ ID 1; 382 amino acids), ANV-6L15 (SEQ ID 2; 378 amino acids), ANV-KAPP (SEQ ID 3; 376 amino acids), and ANV-KKTFPI (SEQ ID 4; 459 amino acids), or their conservatively substituted variants. Preferred Composition: The anti-thrombotic composition further comprises an excipient. Preferred Method: Producing the recombinant anticoagulant protein comprises generating a recombinant DNA molecule comprising a first DNA sequence encoding ANV (SEQ ID 9; 960 nucleic acid sequence) and a second DNA sequence encoding KPI. Preferably, the method comprises generating a DNA sequence selected from TAP-ANV (SEQ ID 5; 1380 nucleotide sequence), ANV-6L15 (SEQ ID 6; 1137 nucleotide sequence), ANV-KAPP (SEQ ID 7; 1131 nucleotide sequence), and ANV-KKTFPI (SEQ ID 8; 1380 nucleotide sequence), or their conservatively substituted variants. All sequences are defined in the specification. Preferred Vector: The recombinant expression vector is in a culture of stably transfected prokaryotic or eukaryotic cells.

ACTIVITY - Cardiант; Antithrombotic; Antiarteriosclerotic. No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - The protein and methods are useful for treating unstable angina, myocardial infarction, sudden cardiac death, ischemic stroke, ruptured aneurysms, atherosclerosis, thalassemia, surgical thrombosis, sickle cell disease, or pulmonary embolism.

EXAMPLE - No examples given. (59 pages)

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FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 09:40:05 ON 25 JAN 2008

L1 1 S SITE(W) TARGETED (W) ANTICOAGULANT?
L2 27787 S "ANNEXIN V"
L3 10651 S (FUS? OR ATTCAH? OR BIND?) AND L2
L4 603 S L3 AND ANTICOAGULANT?
L5 0 S TPI AND L4
L6 5303 S KUNITZ (2W) INHIBITOR?
L7 8 S L4 AND L6
L8 2 DUP REM L7 (6 DUPLICATES REMOVED)

=> s 12 and 16

L9 10 L2 AND L6

=> dup rem 19

PROCESSING COMPLETED FOR L9

L10 4 DUP REM L9 (6 DUPLICATES REMOVED)

=> d 1-4 ibib ab

L10 ANSWER 1 OF 4 HCAPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 2006:437762 HCAPLUS
DOCUMENT NUMBER: 144:466044
TITLE: Gene expression profiling of monocytes in diagnosis of leukemias associated with chromosomal translocations affecting the MLL gene and selection of therapies
INVENTOR(S): Haferlach, Torsten; Dugas, Martin; Kern, Wolfgang; Kohlmann, Alexander; Schnittger, Susanne; Schoch, Claudia
PATENT ASSIGNEE(S): Roche Diagnostics G.m.b.H., Germany; F. Hoffmann-La Roche A.-G.
SOURCE: PCT Int. Appl., 1170 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2006048266	A2	20060511	WO 2005-EP11732	20051103
WO 2006048266	A3	20060824		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				

PRIORITY APPLN. INFO.: US 2004-625673P P 20041104

AB Genes showing changes in levels of expression in monocytes in different forms of leukemia compared to healthy monocytes are identified for use in the rapid diagnosis of the disease and in identification of subtypes that will respond well to certain therapies. In addition to methods of genotyping leukemia, the invention also provides related kits and systems.

L10 ANSWER 2 OF 4 HCPLUS COPYRIGHT 2008 ACS on STN
 ACCESSION NUMBER: 2006:191859 HCPLUS
 DOCUMENT NUMBER: 144:252185
 TITLE: Gene expression profiles in peripheral blood mononuclear cells in determination of the nature and severity of stroke
 INVENTOR(S): Baird, Alison E.; Moore, David F.; Goldin, Ehud
 PATENT ASSIGNEE(S): The Gov. Of the U.S.A as Represented by the Secretary of the Dept. Of Health & Human Services, USA
 SOURCE: U.S. Pat. Appl. Publ., 67 pp., Cont.-in-part of Appl. No. PCT/US05/018744.
 CODEN: USXXCO
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 2
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2006046259	A1	20060302	US 2005-155835	20050617
WO 2005116268	A2	20051208	WO 2005-US18744	20050527
WO 2005116268	A3	20061214		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				

PRIORITY APPLN. INFO.: US 2004-575279P P 20040527
 WO 2005-US18744 A2 20050527

AB A method for rapid and accurate diagnosis of the nature and severity of a stroke by measuring gene expression in peripheral blood mononuclear cells is described. Early diagnosis can be used to predict and prevent possible complications. The genes showing altered levels of expression include those associated with white blood cell activation and differentiation; in response to hypoxia, in vascular repair, and those related to a specific peripheral blood mononuclear cell (PBMC) response to the altered cerebral microenvironment. Also provided are methods of identifying one or more agents that alter the activity (such as the expression) of an ischemic stroke-related mol.

L10 ANSWER 3 OF 4 MEDLINE on STN DUPLICATE 1
 ACCESSION NUMBER: 2005231329 MEDLINE
 DOCUMENT NUMBER: PubMed ID: 15677561
 TITLE: Fusion proteins comprising annexin V and Kunitz protease inhibitors are highly potent thrombogenic site-directed anticoagulants.
 AUTHOR: Chen Hsiu-Hui; Vicente Cristina P; He Li; Tollefson Douglas M; Wun Tze-Chein
 CORPORATE SOURCE: Division of Hematology, Department of Medicine, Washington University School of Medicine, St Louis, MO, USA.
 CONTRACT NUMBER: R01 HL55520 (NHLBI)
 SOURCE: Blood, (2005 May 15) Vol. 105, No. 10, pp. 3902-9.
 Electronic Publication: 2005-01-27.
 Journal code: 7603509. ISSN: 0006-4971.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

(RESEARCH SUPPORT, N.I.H., EXTRAMURAL)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)

LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200506
ENTRY DATE: Entered STN: 4 May 2005
Last Updated on STN: 8 Jun 2005
Entered Medline: 7 Jun 2005

AB The anionic phospholipid, phosphatidyl-L-serine (PS), is sequestered in the inner layer of the plasma membrane in normal cells. Upon injury, activation, and apoptosis, PS becomes exposed on the surfaces of cells and sheds microparticles, which are procoagulant. Coagulation is initiated by formation of a tissue factor/factor VIIa complex on PS-exposed membranes and propagated through the assembly of intrinsic tenase (factor VIIIa/factor IXa), prothrombinase (factor Va/factor Xa), and factor XIa complexes on PS-exposed activated platelets. We constructed a novel series of recombinant anticoagulant fusion proteins by linking annexin V (ANV), a PS-binding protein, to the Kunitz-type protease inhibitor (KPI) domain of tick anticoagulant protein, an aprotinin mutant (6L15), amyloid beta-protein precursor, or tissue factor pathway inhibitor. The resulting ANV-KPI fusion proteins were 6- to 86-fold more active than recombinant tissue factor pathway inhibitor and tick anticoagulant protein in an in vitro tissue factor-initiated clotting assay. The in vivo antithrombotic activities of the most active constructs were 3- to 10-fold higher than that of ANV in a mouse arterial thrombosis model. ANV-KPI fusion proteins represent a new class of anticoagulants that specifically target the anionic membrane-associated coagulation enzyme complexes present at sites of thrombogenesis and are potentially useful as antithrombotic agents.

L10 ANSWER 4 OF 4 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
DUPLICATE 2

ACCESSION NUMBER: 2004-04247 BIOTECHDS
TITLE: New recombinant anticoagulant protein comprising a fusion annexin V (ANV) and Kunitz protease inhibitor (KPI), useful for treating unstable angina, myocardial infarction, aneurysms, atherosclerosis, thalassemia, thrombosis; recombinant fusion protein for use in gene therapy

AUTHOR: WUN T C
PATENT ASSIGNEE: WUN T C
PATENT INFO: WO 2003103577 18 Dec 2003
APPLICATION INFO: WO 2003-US17442 4 Jun 2003
PRIORITY INFO: US 2002-386932 6 Jun 2002; US 2002-386932 6 Jun 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-053570 [05]

AB DERWENT ABSTRACT:
NOVELTY - A recombinant anticoagulant protein comprising a fusion annexin V (ANV) (SEQ ID 10; 319 amino acid sequence defined in the specification) and Kunitz protease inhibitor (KPI), is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) an anti-thrombotic composition comprising the recombinant anticoagulant protein; (2) a method of inhibiting blood coagulation in a mammalian subject by administering the recombinant anticoagulant protein to the subject; (3) a method of producing the recombinant anticoagulant protein by linking ANV and KPI; (4) a method of treating or preventing an excess of thrombotic activity in a subject by administering to the subject the anti-thrombotic composition; (5) a recombinant DNA molecule comprising a first DNA sequence encoding ANV and a second DNA sequence encoding KPI; (6) a host cell comprising the recombinant DNA molecule; (7) a stably transfected cell line expressing the recombinant

anticoagulant protein; (8) a prokaryotic or eukaryotic cell line; (9) a process for preparing a cell line expressing the recombinant anticoagulant protein by stably transfecting a host cell with the recombinant expression vector; and (10) a recombinant expression vector comprising a first nucleotide sequence encoding ANV to Ala mutation of ANV, or its conservatively substituted variants, and a second nucleotide sequence of KPI together with additional sequences capable of directing the synthesis of the recombinant anticoagulant protein.

BIOTECHNOLOGY - Preferred Protein: The recombinant anticoagulant protein comprises a protein sequence selected from TAP-ANV (SEQ ID 1; 382 amino acids), ANV-6L15 (SEQ ID 2; 378 amino acids), ANV-KAPP (SEQ ID 3; 376 amino acids), and ANV-KKTFPI (SEQ ID 4; 459 amino acids), or their conservatively substituted variants. Preferred Composition: The anti-thrombotic composition further comprises an excipient. Preferred Method: Producing the recombinant anticoagulant protein comprises generating a recombinant DNA molecule comprising a first DNA sequence encoding ANV (SEQ ID 9; 960 nucleic acid sequence) and a second DNA sequence encoding KPI. Preferably, the method comprises generating a DNA sequence selected from TAP-ANV (SEQ ID 5; 1380 nucleotide sequence), ANV-6L15 (SEQ ID 6; 1137 nucleotide sequence), ANV-KAPP (SEQ ID 7; 1131 nucleotide sequence), and ANV-KKTFPI (SEQ ID 8; 1380 nucleotide sequence), or their conservatively substituted variants. All sequences are defined in the specification. Preferred Vector: The recombinant expression vector is in a culture of stably transfected prokaryotic or eukaryotic cells.

ACTIVITY - Cardiant; Antithrombotic; Antiarteriosclerotic. No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - The protein and methods are useful for treating unstable angina, myocardial infarction, sudden cardiac death, ischemic stroke, ruptured aneurysms, atherosclerosis, thalassemia, surgical thrombosis, sickle cell disease, or pulmonary embolism.

EXAMPLE - No examples given. (59 pages)

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=> s (amyloid beta-protein precursor? ) or (tick anticoagulant peptide) or "acAP5"
or " acap6" or "antistasin"
L11      7748 (AMYLOID BETA-PROTEIN PRECURSOR? ) OR (TICK ANTICOAGULANT PEPTIDE
) OR "ACAP5" OR " ACAP6" OR "ANTISTASIN"

=> d his

(FILE 'HOME' ENTERED AT 09:39:30 ON 25 JAN 2008)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
LIFESCI' ENTERED AT 09:40:05 ON 25 JAN 2008
L1      1 S SITE(W)TARGETED (W)ANTICOAGULANT?
L2      27787 S "ANNEXIN V"
L3      10651 S (FUS? OR ATTCAH? OR BIND?) AND L2
L4      603 S L3 AND ANTICOAGULANT?
L5      0 S TPI AND L4
L6      5303 S KUNITZ (2W)INHIBITOR?
L7      8 S L4 AND L6
L8      2 DUP REM L7 (6 DUPLICATES REMOVED)
L9      10 S L2 AND L6
L10     4 DUP REM L9 (6 DUPLICATES REMOVED)
L11     7748 S (AMYLOID BETA-PROTEIN PRECURSOR? ) OR (TICK ANTICOAGULANT PEPT
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=> s l2 and l11
L12      5 L2 AND L11
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=> dup rem l12
PROCESSING COMPLETED FOR L12
L13      2 DUP REM L12 (3 DUPLICATES REMOVED)
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=> d 1-2 ibib ab

L13 ANSWER 1 OF 2 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2005231329 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15677561
TITLE: Fusion proteins comprising annexin V
and Kunitz protease inhibitors are highly potent
thrombogenic site-directed anticoagulants.
AUTHOR: Chen Hsiao-Hui; Vicente Cristina P; He Li; Tollesen Douglas
M; Wun Tze-Chein
CORPORATE SOURCE: Division of Hematology, Department of Medicine, Washington
University School of Medicine, St Louis, MO, USA.
CONTRACT NUMBER: R01 HL55520 (NHLBI)
SOURCE: Blood, (2005 May 15) Vol. 105, No. 10, pp. 3902-9.
Electronic Publication: 2005-01-27.
Journal code: 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, N.I.H., EXTRAMURAL)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200506
ENTRY DATE: Entered STN: 4 May 2005
Last Updated on STN: 8 Jun 2005
Entered Medline: 7 Jun 2005

AB The anionic phospholipid, phosphatidyl-L-serine (PS), is sequestered in the inner layer of the plasma membrane in normal cells. Upon injury, activation, and apoptosis, PS becomes exposed on the surfaces of cells and sheds microparticles, which are procoagulant. Coagulation is initiated by formation of a tissue factor/factor VIIa complex on PS-exposed membranes and propagated through the assembly of intrinsic tenase (factor VIIIa/factor IXa), prothrombinase (factor Va/factor Xa), and factor XIa complexes on PS-exposed activated platelets. We constructed a novel series of recombinant anticoagulant fusion proteins by linking annexin V (ANV), a PS-binding protein, to the Kunitz-type protease inhibitor (KPI) domain of tick anticoagulant protein, an aprotinin mutant (6L15), amyloid beta-protein precursor, or tissue factor pathway inhibitor. The resulting ANV-KPI fusion proteins were 6- to 86-fold more active than recombinant tissue factor pathway inhibitor and tick anticoagulant protein in an in vitro tissue factor-initiated clotting assay. The in vivo antithrombotic activities of the most active constructs were 3- to 10-fold higher than that of ANV in a mouse arterial thrombosis model. ANV-KPI fusion proteins represent a new class of anticoagulants that specifically target the anionic membrane-associated coagulation enzyme complexes present at sites of thrombogenesis and are potentially useful as antithrombotic agents.

L13 ANSWER 2 OF 2 MEDLINE on STN
ACCESSION NUMBER: 2001258713 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11223918
TITLE: Calcium ionophore A23187 specifically decreases the secretion of beta-secretase cleaved amyloid precursor protein during apoptosis in primary rat cortical cultures.
AUTHOR: Sennvik K; Benedikz E; Fastbom J; Sundstrom E; Winblad B; Ankarcrona M
CORPORATE SOURCE: Karolinska Institutet, NEUROTEC, Division of Geriatric Medicine, KFC NOVUM, Huddinge, Sweden..
Kristina.Sennvik@kfcmail.hs.sll.se
SOURCE: Journal of neuroscience research, (2001 Mar 1) Vol. 63, No. 5, pp. 429-37.

Journal code: 7600111. ISSN: 0360-4012.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 21 May 2001
Last Updated on STN: 21 May 2001
Entered Medline: 17 May 2001
AB Alzheimer's disease (AD) is characterized by the degeneration and loss of neurons, intracellular neurofibrillary tangles and the accumulation of extracellular senile plaques consisting mainly of beta-amyloid (A beta). A beta is generated from the amyloid precursor protein (APP) by sequential beta- and gamma-secretase cleavage. Alternatively, APP may be cleaved within the A beta region by alpha-secretase, preventing A beta formation. Here we investigated APP processing and secretion in primary neurons, using either colchicine or the calcium ionophore A23187 to induce apoptosis. Cell viability was determined by MTT measurements and apoptosis was further confirmed by annexin V and propidium iodide staining. We found that exposure to A23187 significantly decreased the secretion of soluble beta-secretase cleaved APP (beta-sAPP) in a caspase-dependent manner, although the secretion of total soluble APP beta sAPP) did not change. In addition, caspase inhibition restored cell viability to control levels. Exposure to colchicine did not change the amount of either secreted beta-sAPP or total sAPP and caspase inhibition was only partially able to restore cell viability. We conclude that calcium homeostasis is an important apoptotic effector specifically affecting the beta-secretase cleavage of APP.
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(FILE 'HOME' ENTERED AT 09:39:30 ON 25 JAN 2008)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
LIFESCI' ENTERED AT 09:40:05 ON 25 JAN 2008
L1 1 S SITE(W) TARGETED (W) ANTICOAGULANT?
L2 27787 S "ANNEXIN V"
L3 10651 S (FUS? OR ATTCAH? OR BIND?) AND L2
L4 603 S L3 AND ANTICOAGULANT?
L5 0 S TPI AND L4
L6 5303 S KUNITZ (2W) INHIBITOR?
L7 8 S L4 AND L6
L8 2 DUP REM L7 (6 DUPLICATES REMOVED)
L9 10 S L2 AND L6
L10 4 DUP REM L9 (6 DUPLICATES REMOVED)
L11 7748 S (AMYLOID BETA-PROTEIN PRECURSOR?) OR (TICK ANTICOAGULANT PEPT
L12 5 S L2 AND L11
L13 2 DUP REM L12 (3 DUPLICATES REMOVED)

=> s aprotinin and l2

L14 14 APROTININ AND L2

=> dup rem l14

PROCESSING COMPLETED FOR L14

L15 5 DUP REM L14 (9 DUPLICATES REMOVED)

=> d 1-5 ibib ab

L15 ANSWER 1 OF 5 MEDLINE on STN
ACCESSION NUMBER: 2005231329 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15677561

DUPLICATE 1

TITLE: Fusion proteins comprising annexin V and Kunitz protease inhibitors are highly potent thrombogenic site-directed anticoagulants.

AUTHOR: Chen Hsiu-Hui; Vicente Cristina P; He Li; Tollesen Douglas M; Wun Tze-Chein

CORPORATE SOURCE: Division of Hematology, Department of Medicine, Washington University School of Medicine, St Louis, MO, USA.

CONTRACT NUMBER: R01 HL55520 (NHLBI)

SOURCE: Blood, (2005 May 15) Vol. 105, No. 10, pp. 3902-9.

Electronic Publication: 2005-01-27.

Journal code: 7603509. ISSN: 0006-4971.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, N.I.H., EXTRAMURAL)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)

LANGUAGE: English

FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals

ENTRY MONTH: 200506

ENTRY DATE: Entered STN: 4 May 2005
Last Updated on STN: 8 Jun 2005
Entered Medline: 7 Jun 2005

AB The anionic phospholipid, phosphatidyl-L-serine (PS), is sequestered in the inner layer of the plasma membrane in normal cells. Upon injury, activation, and apoptosis, PS becomes exposed on the surfaces of cells and sheds microparticles, which are procoagulant. Coagulation is initiated by formation of a tissue factor/factor VIIa complex on PS-exposed membranes and propagated through the assembly of intrinsic tenase (factor VIIIa/factor IXa), prothrombinase (factor Va/factor Xa), and factor XIa complexes on PS-exposed activated platelets. We constructed a novel series of recombinant anticoagulant fusion proteins by linking annexin V (ANV), a PS-binding protein, to the Kunitz-type protease inhibitor (KPI) domain of tick anticoagulant protein, an aprotinin mutant (6L15), amyloid beta-protein precursor, or tissue factor pathway inhibitor. The resulting ANV-KPI fusion proteins were 6- to 86-fold more active than recombinant tissue factor pathway inhibitor and tick anticoagulant protein in an in vitro tissue factor-initiated clotting assay. The in vivo antithrombotic activities of the most active constructs were 3- to 10-fold higher than that of ANV in a mouse arterial thrombosis model. ANV-KPI fusion proteins represent a new class of anticoagulants that specifically target the anionic membrane-associated coagulation enzyme complexes present at sites of thrombogenesis and are potentially useful as antithrombotic agents.

L15 ANSWER 2 OF 5 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
ACCESSION NUMBER: 2005:476902 BIOSIS
DOCUMENT NUMBER: PREV200510268806

TITLE: Hemostatic properties of infusible trehalose-stabilized lyophilized platelet derivatives.

AUTHOR(S): Moskowitz, Keith A. [Reprint Author]; Dee, Josh; Barnidge, Jason; Sum, Ruth; Ho, David; Rudolph, Alan S.; Orser, Cindy S.

CORPORATE SOURCE: Adlyfe Inc, Hematol Dept, Rockville, MD USA
SOURCE: Blood, (NOV 16 2004) Vol. 104, No. 11, Part 1, pp. 238A.
Meeting Info.: 46th Annual Meeting of the American-Society-of-Hematology. San Diego, CA, USA.
December 04 -07, 2004. Amer Soc Hematol.
CODEN: BLOOAW. ISSN: 0006-4971.

DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)

LANGUAGE: English

ENTRY DATE: Entered STN: 16 Nov 2005
Last Updated on STN: 16 Nov 2005

AB Availability of platelet concentrates for treatment of bleeding associated

with thrombocytopenia, trauma, or drug-induced coagulopathies is problematic due to the short 5 day platelet storage time and because platelets require controlled shaking at ambient temperature in order to remain viable, a condition which augments bacterial growth. To address the platelet availability problem we expanded upon trehalose cryo-preservation technology to create a lyophilized hemostatic platelet derivative. Washed platelets were stabilized by accumulation of 5-10 mM intracellular trehalose via fluid phase endocytosis then formulated with excipients and lyophilized. Lyophilized platelets were instantaneously rehydrated with > 90% recovery and were stable for at least 3-6 months at ambient temperatures. Rehydrated (RH) platelets responded quantitatively to alpha- and gamma-thrombin and ristocetin by transmittance aggregometry and were partially agglutinated by collagen as judged by aggregometry and single cell counting using the Platelet Works (R) system. RH platelets co-aggregated in a dose dependent manner when mixed with fresh autologous platelets during collagen-induced activation. Aggregation response to low-dose thrombin and collagen was inhibited by the GPIIb/IIIa antagonist RGDS and by EGTA. RH platelets were quantitatively incorporated into fibrin clots and elicited platelet-dependent fibrin-clot retraction similar to 60% as well as fresh platelets. RH platelets were similar in size to fresh and had less than 25% submicron particles as judged by electronic particle counting and flow cytometry scatter profiles. RH platelets were partially activated upon rehydration as judged by anti P-selectin and anti-LAMP-3 binding, yet GPIIb/IIIa remained in a testing conformation, as judged by a lack of PAC-1 binding. GPIIb/IIIa receptors were present as judged by the binding of complex-dependent (clone 5B12) and function-blocking (clone P2) antibodies. RH platelets also contained intact GPIb alpha as judged by binding of the function-blocking MoAb AN51. Function of GPIIb/IIIa and collagen receptors on RH platelets was further demonstrated as RH platelets adhered to immobilized fibrinogen and collagen in the absence of added agonists and in a dose-dependent manner. Moreover, RH platelets exhibited a two-fold increase in platelet procoagulant activity in the presence of thrombin receptor agonist peptide SFLLRN as judged by Annexin-V binding. Procoagulant and hemostatic activity was further demonstrated as RH platelets accelerated the clotting of recalcified whole thrombocytopenic blood in a dose-dependent manner similarly to fresh platelets. Lastly, RH platelets corrected the coagulopathy induced by contact pathway inhibition with aprotinin during the recalcification of citrated whole blood. The technology has been scaled to single donor platelet aphaeresis units, equivalent to a standard transfusion dose. Preclinical animal models of safety, efficacy, and circulation persistence are currently being evaluated. In summary, trehalose- stabilized lyophilized platelet derivatives contain numerous in vitro hemostatic properties and may offer an attractive alternative to fresh platelet transfusions when the latter are indicated yet unavailable.

L15 ANSWER 3 OF 5 HCPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2003:991276 HCPLUS
DOCUMENT NUMBER: 140:35943
TITLE: Recombinant fusion of annexin V
(ANV) and Kunitz protease inhibitors (KPI) as novel
site-targeted anticoagulants exhibiting stronger
activities than their components
INVENTOR(S): Wun, Tze Chein
PATENT ASSIGNEE(S): USA
SOURCE: PCT Int. Appl., 59 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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WO 2003103577	A2	20031218	WO 2003-US17442	20030604
WO 2003103577	A3	20040304		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2486362	A1	20031218	CA 2003-2486362	20030604
AU 2003237351	A1	20031222	AU 2003-237351	20030604
AU 2003237351	A2	20031222		
EP 1509541	A2	20050302	EP 2003-736814	20030604
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
CN 1659180	A	20050824	CN 2003-812999	20030604
JP 2005528124	T	20050922	JP 2004-510698	20030604
US 2005164926	A1	20050728	US 2005-516908	20050317
PRIORITY APPLN. INFO.:			US 2002-386932P	P 20020606
			WO 2003-US17442	W 20030604

AB Novel recombinant anticoagulation proteins, methods of their use and methods of their production are described. In particular, recombinant fusions of annexin V (ANV) and Kunitz protease inhibitors (KPI) that possess potent anticoagulant activity are provided. The fusions, abbreviated ANV:KPI, utilize ANV having high affinity for phosphatidyl-L-serine with various KPI's to target serine proteases in membrane-associated coagulation complexes in the blood coagulation cascade. ANV:KPIs are potentially useful antithrombotic drugs permitting localized passivation of thrombogenic vessel walls and associated thrombi.

L15 ANSWER 4 OF 5	MEDLINE on STN	DUPLICATE 2
ACCESSION NUMBER:	1999441380	MEDLINE
DOCUMENT NUMBER:	PubMed ID: 10510399	
TITLE:	Activated lymphocytes promote endothelial cell detachment from matrix: a role for modulation of endothelial cell beta 1 integrin affinity.	
AUTHOR:	Phan C; McMahon A W; Nelson R C; Elliott J F; Murray A G	
CORPORATE SOURCE:	Department of Medicine, University of Alberta, Edmonton, Canada.	
SOURCE:	Journal of immunology (Baltimore, Md. : 1950), (1999 Oct 15) Vol. 163, No. 8, pp. 4557-63. Journal code: 2985117R. ISSN: 0022-1767.	
PUB. COUNTRY:	United States	
DOCUMENT TYPE:	Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)	
LANGUAGE:	English	
FILE SEGMENT:	Abridged Index Medicus Journals; Priority Journals	
ENTRY MONTH:	199911	
ENTRY DATE:	Entered STN: 11 Jan 2000 Last Updated on STN: 11 Jan 2000 Entered Medline: 4 Nov 1999	

AB In vivo, MHC class I-restricted injury of allogeneic tissue or cells infected by intracellular pathogens occurs in the absence of classical cytolytic effector mechanisms and Ab. Modulation of the target cell adhesion to matrix may be an additional mechanism used to injure vascular or epithelial cells in inflammation. We studied the mechanisms of human umbilical vein endothelial cell (EC) detachment from matrix-coated plastic following contact by concanamycin A-treated lymphocytes as an in vitro model of perforin-independent modulation of EC basement membrane adhesion. Human PBL were depleted of monocytes, stimulated, then added to an EC

monolayer plated on either fibronectin or type I collagen matrices. Activated, but not resting, PBL induced progressive EC detachment from the underlying matrix. Injury of the EC monolayer required direct cell contact with the activated lymphocytes because no detachment was seen when the PBL were placed above a Transwell membrane. Moreover plasma membranes prepared from activated but not resting PBL induced EC detachment. Adherent EC stimulated with activated PBL did not show evidence of apoptosis using TUNEL and annexin V staining at time points before EC detachment was observed. Finally, neither the matrix metalloproteinase inhibitors o-phenanthroline and BB-94 nor aprotinin blocked EC detachment. However, activation of EC beta1 integrin using mAb TS2/16 or Mg²⁺ decreased EC detachment. These data indicate that cell-cell contact between activated PBL and EC reduces adhesion of EC to the underlying matrix, at least in part by inducing changes in the affinity of the endothelial beta 1 integrin.

L15 ANSWER 5 OF 5 EMBASE COPYRIGHT (c) 2008 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 1996075313 EMBASE

TITLE: Surface blebs on apoptotic cells are sites of enhanced procoagulant activity: Implications for coagulation events and antigenic spread in systemic lupus erythematosus.

AUTHOR: Casciola-Rosen L.; Rosen A.; Petri M.; Schlissel M.

CORPORATE SOURCE: L. Casciola-Rosen, Department of Dermatology, Johns Hopkins Univ. Sch. of Medicine, Baltimore, MD 21205, United States

SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (20 Feb 1996) Vol. 93, No. 4, pp. 1624-1629.

Refs: 25

ISSN: 0027-8424 CODEN: PNASA6

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 013 Dermatology and Venereology

026 Immunology, Serology and Transplantation

LANGUAGE: English

SUMMARY LANGUAGE: English

ENTRY DATE: Entered STN: 25 Mar 1996

Last Updated on STN: 25 Mar 1996

AB The restriction of phosphatidylserine (PtdSer) to the inner surface of the plasma membrane bilayer is lost early during apoptosis. Since PtdSer is a potent surface procoagulant, and since there is an increased incidence of coagulation events in patients with systemic lupus erythematosus (SLE) who have anti-phospholipid antibodies, we addressed whether apoptotic cells are procoagulant and whether anti-phospholipid antibodies influence this. Apoptotic HeLa cells, human endothelial cells, and a murine pre-B-cell line were markedly procoagulant in a modified Russell viper venom assay. This procoagulant effect was entirely abolished by addition of the PtdSer-binding protein, annexin V, confirming that it was PtdSer-dependent. The procoagulant effect was also abolished by addition of IgG purified from the plasma of three patients with anti-phospholipid antibody syndrome, but not IgG from normal controls. Confocal microscopy of apoptotic cells stained with fluorescein-isothiocyanate-conjugated-annexin V demonstrated Ca(2+)-dependent binding to the surface of membrane blebs on apoptotic cells, but not to intracellular membranes. Recent data indicate that the surface blebs of apoptotic cells constitute an important immunogenic particle in SLE. We propose that the PtdSer exposed on the outside of these blebs can induce the production of antiphospholipid antibodies, which might also enhance the immunogenicity of the bleb contents. When apoptosis occurs in a microenvironment in direct contact with circulating plasma, the unique procoagulant consequences of the apoptotic surface may additionally be expressed. This might explain the increased incidence of pathological intravascular coagulation events that occur in some lupus patients who have anti-phospholipid antibodies.

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(FILE 'HOME' ENTERED AT 09:39:30 ON 25 JAN 2008)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 09:40:05 ON 25 JAN 2008

L1 1 S SITE(W) TARGETED (W) ANTICOAGULANT?
L2 27787 S "ANNEXIN V"
L3 10651 S (FUS? OR ATTCAH? OR BIND?) AND L2
L4 603 S L3 AND ANTICOAGULANT?
L5 0 S TPI AND L4
L6 5303 S KUNITZ (2W) INHIBITOR?
L7 8 S L4 AND L6
L8 2 DUP REM L7 (6 DUPLICATES REMOVED)
L9 10 S L2 AND L6
L10 4 DUP REM L9 (6 DUPLICATES REMOVED)
L11 7748 S (AMYLOID BETA-PROTEIN PRECURSOR?) OR (TICK ANTICOAGULANT PEPT
L12 5 S L2 AND L11
L13 2 DUP REM L12 (3 DUPLICATES REMOVED)
L14 14 S APROTININ AND L2
L15 5 DUP REM L14 (9 DUPLICATES REMOVED)

=> e wun t c/au

E1 1 WUN SOK PARK/AU
E2 252 WUN T/AU
E3 281 --> WUN T C/AU
E4 2 WUN T C J/AU
E5 5 WUN T H/AU
E6 1 WUN T W/AU
E7 1 WUN T Z/AU
E8 1 WUN TAO/AU
E9 1 WUN TC/AU
E10 100 WUN TED/AU
E11 1 WUN TEN T/AU
E12 1 WUN THEADORE/AU

=> s e3

L16 281 "WUN T C"/AU

=> s 16 and 116

L17 25 L6 AND L16

=> dup rem 117

PROCESSING COMPLETED FOR L17

L18 9 DUP REM L17 (16 DUPLICATES REMOVED)

=> d 1-9 ibib ab

L18 ANSWER 1 OF 9 EMBASE COPYRIGHT (c) 2008 Elsevier B.V. All rights reserved on STN DUPLICATE 1

ACCESSION NUMBER: 2005214557 EMBASE

TITLE: Fusion proteins comprising annexin V and Kunitz protease inhibitors are highly potent thrombogenic site-directed anticoagulants.

AUTHOR: Chen H.-H.; Vicente C.P.; He L.; Tollefson D.M.; Wun T.-C.

CORPORATE SOURCE: T.-C. Wun, EVAS Therapeutics, 613 Huntley Heights Dr, Ballwin, MO 63021, United States. tcwun@hotmail.com

SOURCE: Blood, (15 May 2005) Vol. 105, No. 10, pp. 3902-3909. Refs: 66

ISSN: 0006-4971 CODEN: BLOOAW

COUNTRY: United States

DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 025 Hematology
029 Clinical and Experimental Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 9 Jun 2005
Last Updated on STN: 9 Jun 2005

AB The anionic phospholipid, phosphatidyl-L-serine (PS), is sequestered in the inner layer of the plasma membrane in normal cells. Upon injury, activation, and apoptosis, PS becomes exposed on the surfaces of cells and sheds microparticles, which are procoagulant. Coagulation is initiated by formation of a tissue factor/factor VIIa complex on PS-exposed membranes and propagated through the assembly of intrinsic tenase (factor VIIIa/factor IXa), prothrombinase (factor Va/factor Xa), and factor XIa complexes on PS-exposed activated platelets. We constructed a novel series of recombinant anticoagulant fusion proteins by linking annexin V (ANV), a PS-binding protein, to the Kunitz-type protease inhibitor (KPI) domain of tick anticoagulant protein, an aprotinin mutant (6L15), amyloid β -protein precursor, or tissue factor pathway inhibitor. The resulting ANV-KPI fusion proteins were 6- to 86-fold more active than recombinant tissue factor pathway inhibitor and tick anticoagulant protein in an in vitro tissue factor-initiated clotting assay. The in vivo antithrombotic activities of the most active constructs were 3- to 10-fold higher than that of ANV in a mouse arterial thrombosis model. ANV-KPI fusion proteins represent a new class of anticoagulants that specifically target the anionic membrane-associated coagulation enzyme complexes present at sites of thrombogenesis and are potentially useful as antithrombotic agents. .COPYRGT. 2005 by The American Society of Hematology.

L18 ANSWER 2 OF 9 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-04247 BIOTECHDS

TITLE: New recombinant anticoagulant protein comprising a fusion annexin V (ANV) and Kunitz protease inhibitor (KPI), useful for treating unstable angina, myocardial infarction, aneurysms, atherosclerosis, thalassemia, thrombosis; recombinant fusion protein for use in gene therapy
AUTHOR: WUN T C
PATENT ASSIGNEE: WUN T C
PATENT INFO: WO 2003103577 18 Dec 2003
APPLICATION INFO: WO 2003-US17442 4 Jun 2003
PRIORITY INFO: US 2002-386932 6 Jun 2002; US 2002-386932 6 Jun 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-053570 [05]

AB DERWENT ABSTRACT:
NOVELTY - A recombinant anticoagulant protein comprising a fusion annexin V (ANV) (SEQ ID 10; 319 amino acid sequence defined in the specification) and Kunitz protease inhibitor (KPI), is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) an anti-thrombotic composition comprising the recombinant anticoagulant protein; (2) a method of inhibiting blood coagulation in a mammalian subject by administering the recombinant anticoagulant protein to the subject; (3) a method of producing the recombinant anticoagulant protein by linking ANV and KPI; (4) a method of treating or preventing an excess of thrombotic activity in a subject by administering to the subject the anti-thrombotic composition; (5) a recombinant DNA molecule comprising a first DNA sequence encoding ANV and a second DNA sequence encoding KPI; (6) a host cell comprising the recombinant DNA molecule; (7) a stably transfected cell line expressing the recombinant anticoagulant protein; (8) a prokaryotic or eukaryotic cell line; (9) a process for preparing a cell line expressing the recombinant anticoagulant protein by stably transfecting a host cell with the

recombinant expression vector; and (10) a recombinant expression vector comprising a first nucleotide sequence encoding ANV to Ala mutation of ANV, or its conservatively substituted variants, and a second nucleotide sequence of KPI together with additional sequences capable of directing the synthesis of the recombinant anticoagulant protein.

BIOTECHNOLOGY - Preferred Protein: The recombinant anticoagulant protein comprises a protein sequence selected from TAP-ANV (SEQ ID 1; 382 amino acids), ANV-6L15 (SEQ ID 2; 378 amino acids), ANV-KAPP (SEQ ID 3; 376 amino acids), and ANV-KKTFPI (SEQ ID 4; 459 amino acids), or their conservatively substituted variants. Preferred Composition: The anti-thrombotic composition further comprises an excipient. Preferred Method: Producing the recombinant anticoagulant protein comprises generating a recombinant DNA molecule comprising a first DNA sequence encoding ANV (SEQ ID 9; 960 nucleic acid sequence) and a second DNA sequence encoding KPI. Preferably, the method comprises generating a DNA sequence selected from TAP-ANV (SEQ ID 5; 1380 nucleotide sequence), ANV-6L15 (SEQ ID 6; 1137 nucleotide sequence), ANV-KAPP (SEQ ID 7; 1131 nucleotide sequence), and ANV-KKTFPI (SEQ ID 8; 1380 nucleotide sequence), or their conservatively substituted variants. All sequences are defined in the specification. Preferred Vector: The recombinant expression vector is in a culture of stably transfected prokaryotic or eukaryotic cells.

ACTIVITY - Cardiant; Antithrombotic; Antiarteriosclerotic. No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - The protein and methods are useful for treating unstable angina, myocardial infarction, sudden cardiac death, ischemic stroke, ruptured aneurysms, atherosclerosis, thalassemia, surgical thrombosis, sickle cell disease, or pulmonary embolism.

EXAMPLE - No examples given. (59 pages)

L18 ANSWER 3 OF 9	MEDLINE on STN	DUPLICATE 2
ACCESSION NUMBER:	2002010420 MEDLINE	
DOCUMENT NUMBER:	PubMed ID: 11372676	
TITLE:	Recombinant tissue factor pathway inhibitor enhances the binding of factor Xa to human monocytes.	
AUTHOR:	Li A; Chang A C; Peer G T; Wun T C; Taylor F B Jr	
CORPORATE SOURCE:	Cardiovascular Biology Program, Oklahoma Medical Research Foundation, Oklahoma City 73104, USA.	
SOURCE:	Thrombosis and haemostasis, (2001 May) Vol. 85, No. 5, pp. 830-6.	
PUB. COUNTRY:	Journal code: 7608063. ISSN: 0340-6245.	
DOCUMENT TYPE:	Germany: Germany, Federal Republic of	
LANGUAGE:	Journal; Article; (JOURNAL ARTICLE)	
FILE SEGMENT:	English	
ENTRY MONTH:	Priority Journals	
ENTRY DATE:	200202	
	Entered STN: 21 Jan 2002	
	Last Updated on STN: 20 Feb 2002	
	Entered Medline: 19 Feb 2002	

AB Tissue factor pathway inhibitor (TFPI) is a kunitz-type inhibitor of activated factor X (Xa). TFPI was reported to mediate Xa binding to a few of carcinoma cell lines. In this study it was observed that the Xa activity associated with human peripheral blood mononuclear cells (PBMC) incubated with Xa in the presence of recombinant TFPI (rTFPI) was much higher than with Xa alone. Xa activity on PBMC was also observed after whole blood was incubated with pre-formed Xa/TFPI complex. Further studies with flow cytometric analysis demonstrate that rTFPI enhances the binding of Xa to human monocytes. Western blot analysis showed that rTFPI was cleaved into a few of fragments after its incubation with monocytes either in the presence or absence of Xa. Based on these results and the observations reported by others, we speculate that Xa/TFPI complex may bind to human monocytes by a yet unidentified mechanism. The recovery of Xa activity from Xa/TFPI complex on PBMC may

be related to the cleavage of rTFPI by Xa and/or monocyte proteases. This observation suggests a new mechanism by which monocytes become procoagulant in some pathological conditions in addition of the well known tissue factor expression on proinflammatory monocytes.

L18 ANSWER 4 OF 9 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 1998380009 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9716152
TITLE: Factor Xa cleavage of tissue factor pathway inhibitor is associated with loss of anticoagulant activity.
AUTHOR: Salemink I; Franssen J; Willems G M; Hemker H C; Li A; Wun T C; Lindhout T
CORPORATE SOURCE: Department of Biochemistry, Maastricht University, The Netherlands.
SOURCE: Thrombosis and haemostasis, (1998 Aug) Vol. 80, No. 2, pp. 273-80.
PUB. COUNTRY: Journal code: 7608063. ISSN: 0340-6245.
DOCUMENT TYPE: GERMANY: Germany, Federal Republic of (COMPARATIVE STUDY)
Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 15 Jan 1999
Last Updated on STN: 15 Jan 1999
Entered Medline: 4 Dec 1998

AB Tissue factor:factor VIIa induced activation of blood coagulation is inhibited by the complex between factor Xa and tissue factor pathway inhibitor (factor Xa:TFPI). We recently reported that phospholipid-bound factor Xa reduces the high binding affinity of factor Xa:TFPI for negatively charged phospholipids by a partial degradation of TFPI (17). The present study was undertaken to elucidate the factor Xa cleavage sites in TFPI and to delineate the consequences of this proteolysis with respect to the inhibitory activity of factor Xa:TFPI. We found that phospholipid-bound factor Xa cleaves in TFPI the peptide bonds between Lys86-Thr87 and Arg199-Ala200. Interestingly, Arg199 is the P1 residue of the third Kunitz-type protease inhibitor domain. The fast cleavage of the Arg199-Ala200 bond results in a 50-70% reduction of the anticoagulant activity of factor Xa:TFPI, as determined with a dilute tissue factor assay, but is not associated with a diminished inhibitory activity of factor Xa:TFPI towards TF:factor VIIa catalyzed activation of factor X. On the other hand, the slower cleavage of the Lys86-Thr87 peptide bond was associated with both a diminished anticoagulant and anti-TF:factor VIIa activity. Dissociation of factor Xa from the cleaved TFPI was not observed. These data provide evidence for a dual role of factor Xa since it is the essential cofactor in the TFPI-controlled regulation of TF-dependent coagulation as well as a catalyst of the inactivation of TFPI.

L18 ANSWER 5 OF 9 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 96202546 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8619181
TITLE: Prevention of spinal cord injury after transient aortic clamping with tissue factor pathway inhibitor.
AUTHOR: Koudsi B; Yu C D; Ferguson E W Jr; Miller G A; Merkel K D; Wun T C; Kraemer B A
CORPORATE SOURCE: Department of Orthopaedic Surgery, Washington University School of Medicine, St. Louis, Missouri 63110, USA.
SOURCE: Surgery, (1996 Mar) Vol. 119, No. 3, pp. 269-74.
Journal code: 0417347. ISSN: 0039-6060.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199606
ENTRY DATE: Entered STN: 20 Jun 1996
Last Updated on STN: 20 Jun 1996
Entered Medline: 10 Jun 1996

AB BACKGROUND: Lower limb paralysis that occurs in 11% of patients after treatment of thoracic and thoracoabdominal aortic aneurysms is unpredictable and at present not preventable. The proposed cause for the neurologic changes is believed to be spinal cord ischemia combined with ischemia/reperfusion injury. Recombinant tissue factor pathway inhibitor (rTFPI), a multivalent Kunitz-type inhibitor that binds to tissue factor-VIIa complex, was evaluated. METHODS: The effectiveness of rTFPI as an agent to limit spinal cord ischemia/reperfusion injury was studied in a rabbit spinal cord made ischemic for 20 minutes. rTFPI or phosphate-buffered saline solution (control) was given in randomized blinded fashion at the onset and conclusion of ischemia. Animals underwent neurologic evaluation at 24 hours in a blinded fashion with a modified Tarlov Scale to rate the lower limb paralysis (score of 4 = normal function, score of 0 = complete paralysis). RESULTS: Seventy-five percent of the TFPI-treated animals had Tarlov scores of 3 to 4, whereas only 29% of the animals treated with phosphate-buffered saline solution had such scores ($p < 0.0014$). Spinal cord histologic findings correlated with the neurologic findings. CONCLUSIONS: We believe that TFPI has unique inhibitory properties that make it an effective agent in limiting postoperative paraplegia associated with spinal ischemia.

L18 ANSWER 6 OF 9 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 94086500 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8262929
TITLE: Kinetics of factor Xa inhibition by tissue factor pathway inhibitor.
AUTHOR: Huang Z F; Wun T C; Broze G J Jr
CORPORATE SOURCE: Division of Hematology/Oncology, Jewish Hospital, Washington University Medical Center, St. Louis, Missouri 63110.
CONTRACT NUMBER: HL-34462 (NHLBI)
SOURCE: The Journal of biological chemistry, (1993 Dec 25) Vol. 268, No. 36, pp. 26950-5.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199401
ENTRY DATE: Entered STN: 9 Feb 1994
Last Updated on STN: 3 Feb 1997
Entered Medline: 27 Jan 1994

AB Tissue factor pathway inhibitor is a multivalent, Kunitz-type proteinase inhibitor. It directly inhibits factor Xa and, in a factor Xa-dependent fashion, produces feedback inhibition of the factor VIIa/tissue factor catalytic complex which is responsible for the initiation of coagulation. Human recombinant TFPI (rTFPI) produced in *Escherichia coli* was used to define the kinetic constants describing the human factor Xa:TFPI interaction. The inactivation of factor Xa by *E. coli*-rTFPI is indistinguishable from that of rTFPI produced in mammalian SK-hepatoma cells, suggesting that post-translational modifications such as glycosylation and phosphorylation do not play a major role in the inhibitory process. The slow, tight-binding inhibition of factor Xa follows the scheme: [formula: see text] Where the enzyme (E) and inhibitor (I) form an initial, immediate collision complex (EI) that then isomerizes slowly to a tightened final EI* complex. In the absence of other

additions, the initial K_i ($=k_2/k_1$) and final K_i^* for the inhibition of factor Xa by *E. coli*-rTFPI are 1.24 nM and 26.4 pM, respectively. In the presence of calcium ions (5 mM) the interaction between factor Xa and rTFPI is substantially weaker, with a K_i of 42.7 nM and K_i^* of 85.2 pM. The addition of other components of the prothrombinase complex produces enhanced factor Xa inhibition predominantly through an effect on the initial K_i . In the presence of calcium ions and saturating concentrations of phospholipids and factor Va, the K_i and K_i^* for factor Xa inactivation are 2.04 nM and 52.3 pM. The enhancing effect of heparin on the inhibitory process is concentration dependent and exhibits an optimum, reminiscent of the "template" model for heparin's acceleration of thrombin and factor IXa inhibition by antithrombin III. At optimal concentrations, the major mechanism of heparin action is also a reduction in the K_i of the initial encounter complex between factor Xa and rTFPI.

L18 ANSWER 7 OF 9 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 92223379 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1562726
TITLE: Tissue factor pathway inhibitor: the carboxy-terminus is required for optimal inhibition of factor Xa.
AUTHOR: Wesselschmidt R; Likert K; Girard T; Wun T C; Broze G J Jr
CORPORATE SOURCE: Division of Hematology/Oncology, Jewish Hospital, Washington University Medical Center, St Louis, MO 63110.
CONTRACT NUMBER: HL-14147 (NHLBI)
HL-34462 (NHLBI)
SOURCE: Blood, (1992 Apr 15) Vol. 79, No. 8, pp. 2004-10.
Journal code: 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199205
ENTRY DATE: Entered STN: 7 Jun 1992
Last Updated on STN: 3 Feb 1997
Entered Medline: 15 May 1992
AB Tissue factor pathway inhibitor (TFPI) is a multivalent Kunitz-type protease inhibitor that binds to and inactivates factor Xa directly, and in a factor Xa-dependent fashion inhibits the factor VIIa/tissue factor catalytic complex. TFPI is a slow, tight-binding, competitive, and reversible inhibitor of factor Xa, in which the formation of an initial encounter complex between TFPI and factor Xa is followed by slow isomerization to a final, tightened complex. Wild-type recombinant TFPI (rTFPI), expressed in mouse C127 cells, separates into two forms on heparin-agarose chromatography that elute at 0.3 mol/L and 0.6 mol/L NaCl. Western blot analysis shows that both forms contain the N-terminus of full-length TFPI, but only rTFPI(0.6) is recognized by an antibody directed against the C-terminus. rTFPI(0.3) and rTFPI(0.6) inhibit factor Xa with 1:1 stoichiometry and inhibit factor VIIa/tissue factor equally in an endpoint-type assay. However, rTFPI(0.6) is a more potent inhibitor than rTFPI(0.3) of coagulation in normal plasma induced by either factor Xa or tissue factor. The initial inhibition of factor Xa (less than 5 seconds) produced by rTFPI(0.6) is several-fold greater than that produced by rTFPI(0.3), presumably reflecting a lower K_i of the immediate encounter complex between factor Xa and TFPI. The differential effect of these forms of TFPI on tissue factor-induced coagulation in normal plasma appears to be directly related to their ability to inhibit factor Xa. To confirm the role of the C-terminal region of TFPI in optimal factor Xa inhibition, a carboxy-terminal mutant of rTFPI, which is truncated after leucine 252 and thus lacks the basic sequence K T K R K R K K Q R V K (residues 254-265), was expressed in C127 cells. This form of rTFPI elutes from heparin-agarose at 0.28 mol/L NaCl and inhibits factor Xa at a

rate that is slower than rTFPI(0.3). The K_i (final)s for factor Xa inhibition by rTFPI(0.6), rTFPI(0.3), and rTFPI1-252 are 3.1 ± 0.6 , 19.6 ± 0.8 , and 19.6 ± 3.0 pmol/L, respectively.

L18 ANSWER 8 OF 9 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 92216079 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1558967
TITLE: The effect of leukocyte elastase on tissue factor pathway inhibitor.
AUTHOR: Higuchi D A; Wun T C; Likert K M; Broze G J Jr
CORPORATE SOURCE: Department of Medicine, Jewish Hospital, Washington University Medical Center, St Louis, MO 63110.
CONTRACT NUMBER: HL34462 (NHLBI)
SOURCE: Blood, (1992 Apr 1) Vol. 79, No. 7, pp. 1712-9.
Journal code: 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199205
ENTRY DATE: Entered STN: 29 May 1992
Last Updated on STN: 3 Mar 2000
Entered Medline: 14 May 1992

AB Tissue factor pathway inhibitor (TFPI) is a multivalent Kunitz-type inhibitor that directly inhibits factor Xa and, in a factor Xa-dependent fashion, also inhibits the factor VIIa/tissue factor (TF) catalytic complex. The Kunitz-2 domain in TFPI is needed for the binding and inhibition of factor Xa, while the Kunitz-1 domain appears to be responsible for binding factor VIIa in a quaternary factor Xa-TFPI-factor VIIa/TF inhibitory complex. Human leukocyte elastase (HLE) proteolytically cleaves TFPI between threonine-87 and threonine-88 within the polypeptide that links the Kunitz-1 and Kunitz-2 domains in the TFPI molecule. HLE treatment not only affects the ability of TFPI to inhibit factor VIIa/TF, but also dramatically reduces its inhibition of factor Xa. Both purified HLE and stimulated neutrophils regenerate TF activity from a preformed factor Xa-TFPI-factor VIIa/TF inhibitory complex. Kinetic analysis suggests that HLE cleavage does not effect the affinity of the initial encounter interaction between factor Xa and TFPI, whereas it markedly reduces the affinity of the final factor Xa:TFPI complex with K_i (final) values for untreated and HLE-treated TFPI of 58 pmol/L and 4.4 nmol/L, respectively. Thus, an epitope in the amino-terminal region of TFPI or a conformation of the TFPI molecule that requires the presence of this region is needed in concert with the Kunitz-2 domain to produce optimal inhibition of factor Xa by TFPI.

L18 ANSWER 9 OF 9 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 88198127 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2452157
TITLE: Cloning and characterization of a cDNA coding for the lipoprotein-associated coagulation inhibitor shows that it consists of three tandem Kunitz-type inhibitory domains.
AUTHOR: Wun T C; Kretzmer K K; Girard T J; Miletich J P; Broze G J Jr
CORPORATE SOURCE: Monsanto Co., Chesterfield, Missouri 63198.
CONTRACT NUMBER: HL34462 (NHLBI)
SOURCE: The Journal of biological chemistry, (1988 May 5) Vol. 263, No. 13, pp. 6001-4.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)

(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)

LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-J03225
ENTRY MONTH: 198806
ENTRY DATE: Entered STN: 8 Mar 1990
Last Updated on STN: 3 Mar 2000
Entered Medline: 6 Jun 1988

AB Human plasma contains a lipoprotein-associated coagulation inhibitor (LACI) which inactivates factor Xa directly, and in a Xa-dependent fashion also inhibits the VIIa-tissue factor complex of the extrinsic coagulation pathway. Rabbit polyclonal anti-LACI antiserum was used to screen human placental and fetal liver lambda gt11 cDNA libraries for the expression of LACI antigens. Immunologically positive clones were further tested for their ability to bind ^{125}I -factor Xa. Seven clones were obtained which are immunologically and functionally active. The longest cDNA insert (lambda P9) of these isolates is 1.4 kilobases (kb) while other clones are 1.0 kb in length. Nucleotide sequence analysis shows that lambda P9 consists of 1431 bases that include a 5'-noncoding sequence of 132 nucleotides, an open reading frame of 912 nucleotides, and a 3'-noncoding region of 387 nucleotides. The open reading frame encodes a signal peptide of 28 residues followed by a 32-kilodalton protein of 276 residues. The predicted sequence of mature LACI contains 18 cysteines and three potential N-linked glycosylation sites. The amino acid sequence analysis of purified LACI's NH₂ terminus and two of its proteolytic fragments match exactly those deduced from the cDNA sequence, indicating that the cDNA codes for LACI. The translated amino acid sequence of LACI shows several discernible domains, including a highly negatively charged NH₂ terminus, three tandem Kunitz-type inhibitory domains, and a highly positively charged carboxyl terminus. Northern blot analysis shows that the following liver-derived cell lines, Chang liver, HepG2 hepatoma, and SK hepatoma all, contain two major species of mRNA (1.4 and 4.4 kb) which hybridize with LACI cDNA.

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(FILE 'HOME' ENTERED AT 09:39:30 ON 25 JAN 2008)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCPLUS, NTIS, LIFESCI' ENTERED AT 09:40:05 ON 25 JAN 2008

L1 1 S SITE(W) TARGETED (W) ANTICOAGULANT?
L2 27787 S "ANNEXIN V"
L3 10651 S (FUS? OR ATTCAH? OR BIND?) AND L2
L4 603 S L3 AND ANTICOAGULANT?
L5 0 S TPI AND L4
L6 5303 S KUNITZ (2W) INHIBITOR?
L7 .8 S L4 AND L6
L8 2 DUP REM L7 (6 DUPLICATES REMOVED)
L9 10 S L2 AND L6
L10 4 DUP REM L9 (6 DUPLICATES REMOVED)
L11 7748 S (AMYLOID BETA-PROTEIN PRECURSOR?) OR (TICK ANTICOAGULANT PEPT
L12 5 S L2 AND L11
L13 2 DUP REM L12 (3 DUPLICATES REMOVED)
L14 14 S APROTININ AND L2
L15 5 DUP REM L14 (9 DUPLICATES REMOVED)
E WUN T C/AU
L16 281 S E3
L17 25 S L6 AND L16
L18 9 DUP REM L17 (16 DUPLICATES REMOVED)

=> s l18 ans 12

MISSING OPERATOR L18 ANS

The search profile that was entered contains terms or

nested terms that are not separated by a logical operator.

=> s 118 and 12
L19 2 L18 AND L2

=> d 1-2 ibib ab

L19 ANSWER 1 OF 2 EMBASE COPYRIGHT (c) 2008 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2005214557 EMBASE

TITLE: Fusion proteins comprising annexin V and Kunitz protease inhibitors are highly potent thrombogenic site-directed anticoagulants.

AUTHOR: Chen H.-H.; Vicente C.P.; He L.; Tollefsen D.M.; Wun T.-C.

CORPORATE SOURCE: T.-C. Wun, EVAS Therapeutics, 613 Huntley Heights Dr, Ballwin, MO 63021, United States. tcwun@hotmail.com

SOURCE: Blood, (15 May 2005) Vol. 105, No. 10, pp. 3902-3909.
Refs: 66

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 025 Hematology
029 Clinical and Experimental Biochemistry

LANGUAGE: English

SUMMARY LANGUAGE: English

ENTRY DATE: Entered STN: 9 Jun 2005

Last Updated on STN: 9 Jun 2005

AB The anionic phospholipid, phosphatidyl-L-serine (PS), is sequestered in the inner layer of the plasma membrane in normal cells. Upon injury, activation, and apoptosis, PS becomes exposed on the surfaces of cells and sheds microparticles, which are procoagulant. Coagulation is initiated by formation of a tissue factor/factor VIIa complex on PS-exposed membranes and propagated through the assembly of intrinsic tenase (factor VIIIa/factor IXa), prothrombinase (factor Va/factor Xa), and factor XIa complexes on PS-exposed activated platelets. We constructed a novel series of recombinant anticoagulant fusion proteins by linking annexin V (ANV), a PS-binding protein, to the Kunitz-type protease inhibitor (KPI) domain of tick anticoagulant protein, an aprotinin mutant (6L15), amyloid β -protein precursor, or tissue factor pathway inhibitor. The resulting ANV-KPI fusion proteins were 6- to 86-fold more active than recombinant tissue factor pathway inhibitor and tick anticoagulant protein in an in vitro tissue factor-initiated clotting assay. The in vivo antithrombotic activities of the most active constructs were 3- to 10-fold higher than that of ANV in a mouse arterial thrombosis model. ANV-KPI fusion proteins represent a new class of anticoagulants that specifically target the anionic membrane-associated coagulation enzyme complexes present at sites of thrombogenesis and are potentially useful as antithrombotic agents.

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L19 ANSWER 2 OF 2 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2004-04247 BIOTECHDS

TITLE: New recombinant anticoagulant protein comprising a fusion annexin V (ANV) and Kunitz protease inhibitor (KPI), useful for treating unstable angina, myocardial infarction, aneurysms, atherosclerosis, thalassemia, thrombosis, recombinant fusion protein for use in gene therapy

AUTHOR: WUN T C

PATENT ASSIGNEE: WUN T C

PATENT INFO: WO 2003103577 18 Dec 2003

APPLICATION INFO: WO 2003-US17442 4 Jun 2003

PRIORITY INFO: US 2002-386932 6 Jun 2002; US 2002-386932 6 Jun 2002

DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-053570 [05]

AB DERWENT ABSTRACT:

NOVELTY - A recombinant anticoagulant protein comprising a fusion annexin V (ANV) (SEQ ID 10; 319 amino acid sequence defined in the specification) and Kunitz protease inhibitor (KPI), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) an anti-thrombotic composition comprising the recombinant anticoagulant protein; (2) a method of inhibiting blood coagulation in a mammalian subject by administering the recombinant anticoagulant protein to the subject; (3) a method of producing the recombinant anticoagulant protein by linking ANV and KPI; (4) a method of treating or preventing an excess of thrombotic activity in a subject by administering to the subject the anti-thrombotic composition; (5) a recombinant DNA molecule comprising a first DNA sequence encoding ANV and a second DNA sequence encoding KPI; (6) a host cell comprising the recombinant DNA molecule; (7) a stably transfected cell line expressing the recombinant anticoagulant protein; (8) a prokaryotic or eukaryotic cell line; (9) a process for preparing a cell line expressing the recombinant anticoagulant protein by stably transfecting a host cell with the recombinant expression vector; and (10) a recombinant expression vector comprising a first nucleotide sequence encoding ANV to Ala mutation of ANV, or its conservatively substituted variants, and a second nucleotide sequence of KPI together with additional sequences capable of directing the synthesis of the recombinant anticoagulant protein.

BIOTECHNOLOGY - Preferred Protein: The recombinant anticoagulant protein comprises a protein sequence selected from TAP-ANV (SEQ ID 1; 382 amino acids), ANV-6L15 (SEQ ID 2; 378 amino acids), ANV-KAPP (SEQ ID 3; 376 amino acids), and ANV-KKTFPI (SEQ ID 4; 459 amino acids), or their conservatively substituted variants. Preferred Composition: The anti-thrombotic composition further comprises an excipient. Preferred Method: Producing the recombinant anticoagulant protein comprises generating a recombinant DNA molecule comprising a first DNA sequence encoding ANV (SEQ ID 9; 960 nucleic acid sequence) and a second DNA sequence encoding KPI. Preferably, the method comprises generating a DNA sequence selected from TAP-ANV (SEQ ID 5; 1380 nucleotide sequence), ANV-6L15 (SEQ ID 6; 1137 nucleotide sequence), ANV-KAPP (SEQ ID 7; 1131 nucleotide sequence), and ANV-KKTFPI (SEQ ID 8; 1380 nucleotide sequence), or their conservatively substituted variants. All sequences are defined in the specification. Preferred Vector: The recombinant expression vector is in a culture of stably transfected prokaryotic or eukaryotic cells.

ACTIVITY - Cardiant; Antithrombotic; Antiarteriosclerotic. No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - The protein and methods are useful for treating unstable angina, myocardial infarction, sudden cardiac death, ischemic stroke, ruptured aneurysms, atherosclerosis, thalassemia, surgical thrombosis, sickle cell disease, or pulmonary embolism.

EXAMPLE - No examples given. (59 pages)

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L19 2 S L18 AND L2

=> s l18 and membrane?
L20 1 L18 AND MEMBRANE?

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L20 ANSWER 1 OF 1 EMBASE COPYRIGHT (c) 2008 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2005214557 EMBASE

TITLE: Fusion proteins comprising annexin V and Kunitz protease inhibitors are highly potent thrombogenic site-directed anticoagulants.

AUTHOR: Chen H.-H.; Vicente C.P.; He L.; Tollefsen D.M.; Wun T.-C.

CORPORATE SOURCE: T.-C. Wun, EVAS Therapeutics, 613 Huntley Heights Dr, Ballwin, MO 63021, United States. tcwun@hotmail.com

SOURCE: Blood, (15 May 2005) Vol. 105, No. 10, pp. 3902-3909.
Refs: 66

ISSN: 0006-4971 CODEN: BLOOAW

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 025 Hematology

029 Clinical and Experimental Biochemistry

LANGUAGE: English

SUMMARY LANGUAGE: English

ENTRY DATE: Entered STN: 9 Jun 2005

Last Updated on STN: 9 Jun 2005

AB The anionic phospholipid, phosphatidyl-L-serine (PS), is sequestered in the inner layer of the plasma membrane in normal cells. Upon injury, activation, and apoptosis, PS becomes exposed on the surfaces of cells and sheds microparticles, which are procoagulant. Coagulation is initiated by formation of a tissue factor/factor VIIa complex on PS-exposed membranes and propagated through the assembly of intrinsic tenase (factor VIIa/factor IXa), prothrombinase (factor Va/factor Xa), and factor XIa complexes on PS-exposed activated platelets. We constructed a novel series of recombinant anticoagulant fusion proteins by linking annexin V (ANV), a PS-binding protein, to the Kunitz-type protease inhibitor (KPI) domain of tick anticoagulant protein, an aprotinin mutant (6L15), amyloid β -protein precursor, or tissue factor pathway inhibitor. The resulting ANV-KPI fusion proteins were 6- to 86-fold more active than recombinant tissue factor pathway inhibitor and tick anticoagulant protein in an in vitro tissue factor-initiated clotting assay. The in vivo antithrombotic activities of the most active constructs were 3- to 10-fold higher than that of ANV in a mouse arterial thrombosis model. ANV-KPI fusion proteins represent a new class of anticoagulants that specifically target the anionic membrane-associated coagulation enzyme complexes present at sites of thrombogenesis and are potentially useful as antithrombotic agents.

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L9 10 S L2 AND L6
L10 4 DUP REM L9 (6 DUPLICATES REMOVED)
L11 7748 S (AMYLOID BETA-PROTEIN PRECURSOR?) OR (TICK ANTICOAGULANT PEPT
L12 5 S L2 AND L11
L13 2 DUP REM L12 (3 DUPLICATES REMOVED)
L14 14 S APROTININ AND L2
L15 5 DUP REM L14 (9 DUPLICATES REMOVED)
E WUN T C/AU
L16 281 S E3
L17 25 S L6 AND L16
L18 9 DUP REM L17 (16 DUPLICATES REMOVED)
L19 2 S L18 AND L2
L20 1 S L18 AND MEMBRANE?

=> s (ps (w)binding) and kunitz
L21 6 (PS (W) BINDING) AND KUNITZ

=> dup rem l21
PROCESSING COMPLETED FOR L21
L22 1 DUP REM L21 (5 DUPLICATES REMOVED)

=> d ibib ab

L22 ANSWER 1 OF 1 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2005231329 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15677561
TITLE: Fusion proteins comprising annexin V and Kunitz
protease inhibitors are highly potent thrombogenic
site-directed anticoagulants.
AUTHOR: Chen Hsiao-Hui; Vicente Cristina P; He Li; Tollefson Douglas
M; Wun Tze-Chein
CORPORATE SOURCE: Division of Hematology, Department of Medicine, Washington
University School of Medicine, St Louis, MO, USA.
CONTRACT NUMBER: R01 HL55520 (NHLBI)
SOURCE: Blood, (2005 May 15) Vol. 105, No. 10, pp. 3902-9.
Electronic Publication: 2005-01-27.
Journal code: 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, N.I.H., EXTRAMURAL)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200506
ENTRY DATE: Entered STN: 4 May 2005
Last Updated on STN: 8 Jun 2005

Entered Medline: 7 Jun 2005

AB The anionic phospholipid, phosphatidyl-L-serine (PS), is sequestered in the inner layer of the plasma membrane in normal cells. Upon injury, activation, and apoptosis, PS becomes exposed on the surfaces of cells and sheds microparticles, which are procoagulant. Coagulation is initiated by formation of a tissue factor/factor VIIa complex on PS-exposed membranes and propagated through the assembly of intrinsic tenase (factor VIIIa/factor IXa), prothrombinase (factor Va/factor Xa), and factor XIa complexes on PS-exposed activated platelets. We constructed a novel series of recombinant anticoagulant fusion proteins by linking annexin V (ANV), a PS-binding protein, to the Kunitz-type protease inhibitor (KPI) domain of tick anticoagulant protein, an aprotinin mutant (6L15), amyloid beta-protein precursor, or tissue factor pathway inhibitor. The resulting ANV-KPI fusion proteins were 6- to 86-fold more active than recombinant tissue factor pathway inhibitor and tick anticoagulant protein in an in vitro tissue factor-initiated clotting assay. The in vivo antithrombotic activities of the most active constructs were 3- to 10-fold higher than that of ANV in a mouse arterial thrombosis model. ANV-KPI fusion proteins represent a new class of anticoagulants that specifically target the anionic membrane-associated coagulation enzyme complexes present at sites of thrombogenesis and are potentially useful as antithrombotic agents.

=>
=> d his

(FILE 'HOME' ENTERED AT 09:39:30 ON 25 JAN 2008)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCPLUS, NTIS, LIFESCI' ENTERED AT 09:40:05 ON 25 JAN 2008

L1 1 S SITE(W) TARGETED (W) ANTICOAGULANT?
L2 27787 S "ANNEXIN V"
L3 10651 S (FUS? OR ATTCAH? OR BIND?) AND L2
L4 603 S L3 AND ANTICOAGULANT?
L5 0 S TPI AND L4
L6 5303 S KUNITZ (2W) INHIBITOR?
L7 8 S L4 AND L6
L8 2 DUP REM L7 (6 DUPLICATES REMOVED)
L9 10 S L2 AND L6
L10 4 DUP REM L9 (6 DUPLICATES REMOVED)
L11 7748 S (AMYLOID BETA-PROTEIN PRECURSOR?) OR (TICK ANTICOAGULANT PEPT
L12 5 S L2 AND L11
L13 2 DUP REM L12 (3 DUPLICATES REMOVED)
L14 14 S APROTININ AND L2
L15 5 DUP REM L14 (9 DUPLICATES REMOVED)
E WUN T C/AU
L16 281 S E3
L17 25 S L6 AND L16
L18 9 DUP REM L17 (16 DUPLICATES REMOVED)
L19 2 S L18 AND L2
L20 1 S L18 AND MEMBRANE?
L21 6 S (PS (W) BINDING) AND KUNITZ
L22 1 DUP REM L21 (5 DUPLICATES REMOVED)

	Document ID	Issue Date	Pages	Title
1	US 20070065415 A1	20070322	74	Compositions and methods for the augmentation and repair of defects in tissue
2	US 20070015705 A1	20070118	78	Modified annexin proteins and methods for their use in platelet storage and transfusion
3	US 20050164926 A1	20050728	34	Novel recombinant anticoagulant proteins

	Document ID	Issue Date	Pages	Title
1	US 20070298041 A1	20071227	66	Ligands That Enhance Endogenous Compounds
2	US 20070065415 A1	20070322	74	Compositions and methods for the augmentation and repair of defects in tissue
3	US 20070015705 A1	20070118	78	Modified annexin proteins and methods for their use in platelet storage and transfusion
4	US 20060147451 A1	20060706	75	Modulators of hepatocyte growth factor activator
5	US 20060019893 A1	20060126	27	Factor VIIa variants
6	US 20050164926 A1	20050728	34	Novel recombinant anticoagulant proteins
7	US 20050100991 A1	20050512	184	Albumin fusion proteins
8	US 20030219875 A1	20031127	176	Albumin fusion proteins
9	US 6905688 B2	20050614	208	Albumin fusion proteins
10	US 6423316 B1	20020723	51	Anticoagulant fusion protein anchored to cell membrane
11	US 5312736 A	19940517	24	Anticoagulant analogues of the tissue factor extrinsic pathway inhibitor (EPI) with reduced affinity for heparin

US 20070259417 A1	US-PGPUB	US 5849703 A	USPAT
US 20070020252 A1	US-PGPUB	US 5837500 A	USPAT
US 20060264603 A1	US-PGPUB	US 5824644 A	USPAT
US 20060084113 A1	US-PGPUB	US 5773251 A	USPAT
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US 20020150881 A1	US-PGPUB	US 4966852 A	USPAT
US 20010029034 A1	US-PGPUB		
US 7276480 B1		USPAT	
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US 6548262 B2		USPAT	
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US 6214333 B1		USPAT	
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US 6060449 A		USPAT	
US 6057287 A		USPAT	
US RE36476 E		USPAT	
US 5914316 A		USPAT	
US 5914315 A		USPAT	
US 5849875 A		USPAT	

EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	23836	anticoagulant\$2	US-PGPUB; USPAT	OR	OFF	2008/01/25 10:35
L2	3636	"annexin V"	US-PGPUB; USPAT	OR	OFF	2008/01/25 10:35
L3	747	kunitz adj2 inhibitor\$2	US-PGPUB; USPAT	OR	OFF	2008/01/25 10:36
L4	3	l2 same l3	US-PGPUB; USPAT	OR	OFF	2008/01/25 10:36
L5	173	l3 same (fus\$3 or attach\$3 or bind\$3)	US-PGPUB; USPAT	OR	OFF	2008/01/25 10:38
L6	11	l1 same l5	US-PGPUB; USPAT	OR	OFF	2008/01/25 10:38
L7	677	WUN	US-PGPUB; USPAT	OR	OFF	2008/01/25 10:41
L8	76	l3 and l7	US-PGPUB; USPAT	OR	OFF	2008/01/25 10:41
L9	1	l2 and l8	US-PGPUB; USPAT	OR	OFF	2008/01/25 10:41